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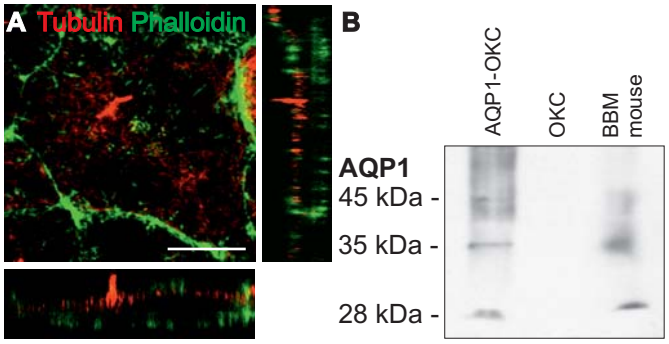
Supplemental data

Supplemental Figure 1. Characterization of opossum kidney cell line stably transfected with rat AQP1 (AQP1-OKC). (A) AQP1-OKC form cilia and microvilli as demonstrated by staining of microtubules (red) and actin filaments (green). Magnification: scale bar = 10 μm. (B) Western blot of AQP1-OKC (lane 1), untransfected OK cells (OKC; lane 2), and mouse kidney BBM fraction (lane 3). Typical AQP1 signal is observed in lane 1 and 3 at 28 kDa (unglycosylated AQP1) and 35-50 kDa (glycosylated AQP1).

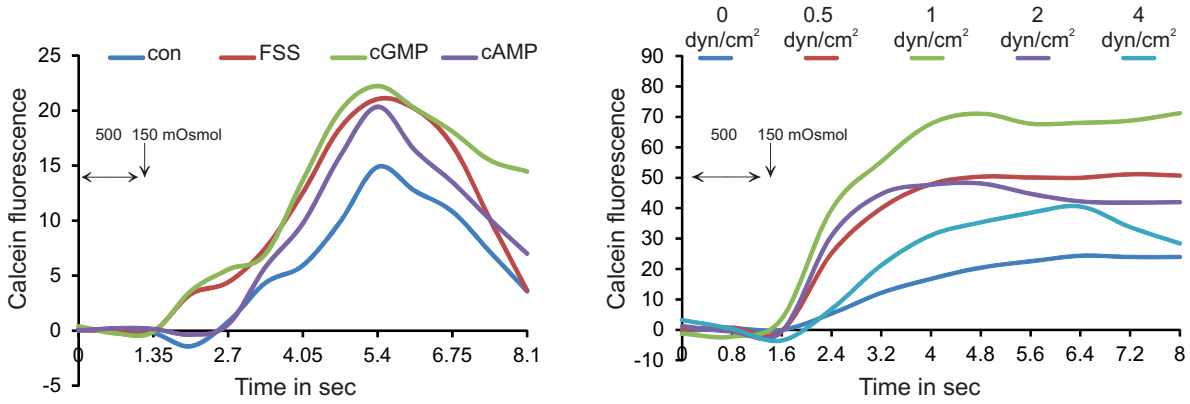
Supplemental Figure 2. Calcein fluorescence quenching. (Left) Calcein-loaded AQP1-OKC were stimulated for 1 hour with FSS, 8-bromo-cGMP or 8-bromo-cAMP, and calcein fluorescence was monitored. Representative curves show the time-course of changes in calcein fluorescence in response to a switch in solution osmolality from 500 to 150 mOsmol. (Right) AQP1-OKC grown in flow chambers were calcein-loaded and various rates of fluid-shear stress were applied for 1h. Representative curves showing the time-course of changes in calcein fluorescence in response to a switch in solution osmolality from 500 to 150 mOsmol.

Supplemental Table 1. AQP1 mRNA abundance. AQP1 mRNA was analyzed by real time PCR. ΔΔCT values of AQP1 and the house keeping gene, GAPDH, of $Lrp2^{fl/fl}$;$apoE^{Cre}$, $Clcn5^{fl/y}$;$villin^{Cre}$, $Clcn5^{+/y}$, and $Cclcn5^{-/y}$ mice are shown. Values are presented as means ± SD; *n* = 6. AQP1 mRNA is not different between groups.

Supplemental Figure 1



Supplemental Figure 2



Supplemental Table 1

	Calculations of AQP1 mRNA ΔΔCT
$Lrp2^{fl/fl}$ vs. $Lrp2^{fl/fl}$;$apoE^{Cre}$	0 ± 1.2% vs. –0.02 ± 0.8%
$Clcn5^{fl/y}$ vs. $Clcn5^{fl/y}$;$villin^{Cre}$	0 ± 0.3% vs. –0.04 ± 0.26%
$Clcn5^{+/y}$ vs. $Clcn5^{-/y}$	0 ± 0.2% vs. 0 ± 0.26%